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Optimization of Lipase Production by *Bacillus megaterium*

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Authors' contributions

This work was carried out in collaboration between both authors. Author FIF designed the study, wrote the protocol and the first draft of the manuscript, read the manuscript for correctness and editing. Author AQP managed the analyses of the study, literature searches and performed the statistical analysis. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To optimize lipase production by *Bacillus megaterium* in submerged fermentation.
Study Design: Collection of palm oil press fibres and effluent from different palm oil mills located within Ibadan Municipality. Isolation of *Bacillus megaterium* by cultivation in medium, submerged fermentation of palm oil press fibres and effluent by *B.* megaterium to produce lipase. Alteration of the cultural conditions to optimize production.
Place and Duration of Study: All work were done in the Department of Microbiology, Faculty of Science, University of Ibadan, from January–December 2014.
Methodology: Palm oil press fibres and effluent were collected from various palm oil mills and were used as the source of isolation of microorganism. The isolated species were identified by studying the morphological, biochemical, characteristics and 16SrNA gene sequencing. The selected species was screened for lipase production.
Results: The results obtained revealed that maximum lipase production was recorded at pH 7.0 with an activity 2.13±0.15 U/ml while the best temperature that supported the optimum production of

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lipase was seen at 35℃ with an activity of 3.30±0.10 U/ml and the best carbon and nitrogen sources were 2% glucose and 2.5% peptone concentrations showing activities of 1.83±0.05 U/ml and 2.60±0.10 U/ml respectively. An incubation period of 72 hours produced the optimum lipase with an activity of 3.26±0.05 U/ml. The separate additions of 0.3M Ca^{2+} and 0.3M Cl^- supported maximum production of lipase.

Conclusion: This study showed that lipase production by *B. megaterium* can be optimized and the best conditions for optimization included pH 7.0, temperature of 35℃, 72 hours incubation period in the presence of 2% glucose, 2.5% peptone concentrations and 0.3M Ca^{2+} and 0.3M Cl^-.

Keywords: Lipase; optimization; Bacillus megaterium; palm oil fibres.

1. INTRODUCTION

Lipases are enzymes that can be derived from animal, plant and microbial sources. They possess enormous bio-catalytic abilities and initiates series of reactions such as hydrolysis, esterification, alchoholysis and aminolysis [1]. Generally, microbial lipases are neutral or alkaline in nature and their thermal stability ranges from 20℃ to 60℃. Stability of lipases in organic solvents is desirable in synthetic reaction and most microbial lipases are stable in organic solvents [2]. They are noted for their vast genetic manipulatory tendency, regular availability and inexpensive cost of production [3-5]. Microorganisms such as yeast, bacteria and fungi have been explored for the production of extracellular lipase in growth medium in the presence of inducers [5,6]. Its production can be made cheaper by employing agricultural waste inducers [7]. It is important to add that lipase activity and production are dependent on the microbial strain and the fermentation medium [5, 8]. Both submerged and solid state fermentations had been reported as possible processes employed in the production of lipolytic enzyme. In addition, the titrimetric method for assaying lipase is widely used because of its simplicity, accuracy, high sensitive and reproducibility [9]. *Bacillus megaterium* is rod-like, Gram-positive and aerobic spore forming bacterium found in diverse habitats. It grows at temperatures from 3℃ to 45℃, with the optimum at 30℃ [10,11].

Lipases of *Bacillus* species origin are important due to the possession of special protein sequences and various outstanding biochemical properties [12]. Bacterial lipases are extracellular enzymes which are mostly released outside the cell and are influenced by nutritional and physico-chemical factors such as temperature, pH, nitrogen and carbon sources, presence of lipids, inorganic salts, stirring conditions, dissolved oxygen concentration [13]. It had been reported to catalyze reversibly the hydrolysis of complex oily based polymer and complex fatty substances to simple fatty acids especially at water – oil interface [2]. Lipases (triacylglycerol acylhydrolases EC.3.1.1.3) belong to a class of hydrolases, they are substrate specific, stereo-specific and thermostable in organic solvent. They exhibit ethanol selectivity and are stable at extreme pH and temperature [14-16]. These attributes of the enzyme makes them suitable to be used in chemical and pharmaceutical industries [17]. The enormous industrial applications of lipase in the dairy, surfactant, food processing, cosmetic , detergent, leather, pharmaceutical and chemical industries is well documented [14,15,18]. According to Rahman et al. [3], a total of 20 million USS Dollars was reported to purchase lipase in the global industrial enzyme market in 2005. Thus, this infers that there is a huge demand for this enzyme because of its wide applications in industrial sectors. This paper attempts to optimize lipase production by *Bacillus megaterium* using the submerged fermentation of agro waste product.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Samples of oil press fibers and effluents were obtained from different palm oil mills located within Ibadan metropolis in Nigeria and brought in sterile flasks to the laboratory for subsequent use.

2.2 Isolation Procedure of Micro-organisms

Ten g of the sample was weighed and aseptically suspended in 90mls of sterile distilled water and was shaken properly. Serial dilution was carried out using the method reported by Meyll and Meynell [19] to obtain dilutions 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}. 0.5 ml of each dilution was differently transferred into sterile several Petri-dishes and

covered with 15 mls of nutrient agar. The plates were incubated at 37℃ for 48h and observed for growth. The isolates obtained were repeatedly streaked to obtain pure cultures which were maintained on nutrient agar slant in McCartney bottles and stored at 4℃ in the refrigeration for subsequent use.

2.3 Screening for Lipolytic Isolates

The pure cultures of isolates obtained were screened for lipolytic activity by employing tributyrin agar which is composed of yeast extract 3.0 g, peptone 5.0 g, NaCl 5.0 g, $CaCl_2$ 0.05 g, glycerol tributyrate 0.2 ml and 20g of agar dissolved in 1 L of distilled water as described by Chatravedi et al. [20]. 0.5 ml of cell suspension of the isolates was differently inoculated into 15 ml of the medium in different Petri dishes and incubation was carried out for 48 h. Lipolytic microorganisms were selected, transferred separately on agar slants and the isolate with the widest zone of clearance was selected for further studies.

2.4 Identification Procedures

The isolate showing the widest zone of clearance was selected, identified using morphological, physiological and biochemical characteristics with reference to Sneath [21] and further confirmation of the isolates identity was performed using 16S rRNA gene sequencing.

2.5 DNA Extraction

Genomic DNA was extracted from a 24 hour old pure bacterial cells suspension as described by Gomma and Montaz [22].

2.6 PCR Amplification of Bacterial 16S rDNA

Oligonucleotide primers were used to amplify 16S rRNA according to the method described by Hengstmann et al. [23].

2.7 Gene Sequencing

The procedure described by Gomma and Montaz [22] was adopted for the Gene sequencing.

2.8 Phylogenetic Analysis

The 16S rDNA sequences derived were incorporated to publicly available bacterial 16S rRNA sequences, the sequences were integrated into the database with the automatic alignment tool. Generation of Phylogenetic tree was done by performing distance matrix analysis using the NT system. Database comparisons were done with the BLAST search using the National Centre for Biotechnology Information (NCBI) database.

2.9 Determination of Inoculum Size

This was determined by transferring a loopful of 24 h old culture of B*.megaterium* into 10 ml of peptone water in a test tube and shaken thoroughly. 1 ml of the bacterial cells suspension was introduced into another test tube containing 0.5ml of crystal violet solution and transferred into a Neubauer counting chamber to enumerate the bacterial cell with the aid of a light microscope x 100.

2.10 Lipase Production

The production medium used contained NaH_2PO_4(12.8 g), KH_2PO_4(3.0 g), NaCl(0.5 g), $MgSO_4.7H_2O$ (0.5 g), NH_4Cl(1.0 g), Glucose(2.0 g) and Castor oil (10 ml) in 1 L of distilled water. 150 ml of the medium was dispensed into 250 ml Erlenmeyer flask and 1 ml of 24 h old bacterial cells ($1.0x10^4$) suspension of the selected isolate was used to inoculate the flask and incubated in a shaker incubator set at 150 rpm for 72 h at 37℃. The medium was centrifuged at 6000 rpm for 30 mins to obtain the supernatant which was used for enzyme assay.

2.11 Lipase Assay

Lipase Activity was determined using the titrimetric method described by Kambiz et al. [24] using castor oil emulsion as substrate. 1 unit of enzyme is defined as the amount of enzyme required to hydrolysis µmol of fatty acid from triglycerides.

2.12 Determination of Optimum pH for Lipase Production

The synthetic medium was sterilized as described earlier and differently adjusted to pH 5.0, 6.0, 7.0, 8.0 and 9.0 with 0.1 M HCl and 0.1M NaOH. 0.5 ml ($1.0x10^4$cfu/ml) of 24 h old culture suspension of B *megaterium* was inoculated into 50 ml of the synthetic medium in 250 ml Erlenmeyer flask, incubated for 37℃ for 72 h and centrifuged at 10000 rpm for 15

minutes. The supernatant obtained was used for enzyme assay.

2.13 Determination of Optimum Temperature for Lipase Production

50 ml of the synthetic medium was dispensed separately into four 250 ml Erlenmeyer flasks and inoculated separately with 0.5 ml ($1.0x10^4$cfu/ml) of 24 h old culture suspension of *B*. *megaterium* and incubated differently at 25℃, 30℃, 35℃ and 40℃ for 72 hours and centrifuged at 10000 rpm for 15 minutes. The resulting supernatant was used for enzyme assay.

2.14 Effect of Different Carbon Sources on Lipase Production

The carbon sources used for this study were Glucose, maltose, lactose, fructose, sucrose, and starch. Glucose was omitted in the composition of the synthetic medium which was sterilized at 121℃ for 15 min and allowed to cool and 1% of the used carbon sources was weighed, sterilized using Millipore filter membrane before separate addition to synthetic medium. Inoculation and incubation of the bacterial cells were carried out as previously described and the obtained supernatant was used for enzyme assay.

2.15 Effect of Different Nitrogen Source on Lipase Production

The nitrogen sources used in this investigation were tryptone, peptone, yeast extract, KNO_3, $(NH_4)2SO_4$, they were supplemented at 1% in the production medium. Inoculation, incubation of the bacterial cells were carried out as previously described and the obtained supernatant used for enzyme assay.

2.16 Determination of Optimum Incubation Period for Lipase Production

0.5 ml ($1.0x10^4$cfu/ml) of 24 h old culture suspensions of B. *megaterium* was inoculated separately into 150 ml of production medium in different four 250 ml Erlenmeyer flasks and incubated at temperatures of 37℃ for 24 h, 48 h, 72 h and 96 h one after the other. The broth culture was centrifuged at 10000 rpm for 15 minutes and the supernatant used for enzyme assay.

2.17 Effect of Different Ions on Lipase Production

The effect of different ions on production was measured by assaying residual activities after the incubation of the enzyme with various concentrations of 5 mM, 10 mM, and 15 mM of the metal ions. The Metal ions used included NaCl, KNO_3, CaCl, $MgSO_4$, $MnSO_4$, NH_4Cl, $FeSO_4$, $NaNO_3$, $NH4NO_3$, $FeCl_2$ and the production medium was employed.

3. RESULTS AND DISCUSSION

3.1 Screening of Bacterial Isolates from Oil Press Fibre for Lipolytic Activities

The results of the screening for lipolytic activity of bacterial isolates obtained from oil press fibre is presented in Table 1. Nine isolates exhibited lipolytic activity on tributyrin agar plate and isolate PFE2 showed the widest zone of clearance (22 mm) after 72 hours of incubation and was selected for use in this study. This isolate was identified based on morphological, biochemical and 16S rRNA gene sequencing as *B. megaterium.* Several researchers had previously reported screening results for microorganisms with abilities for lipolytic activity. Damasco et al. [25] reported the isolation and screening of two stains of fungi from contaminated butters for lipolytic ability and *Aspergillus niger* mutant IIT33A14 was seen as producing the highest zone of clearance. In addition, the screening results of 34 fungal isolates obtained from different oil substrates from raw petrol sample was reported by Adinarayana et al. [26], while Massadeh et al. [27] screened a locally isolated *Bacillus steareothermophilus* HUI from petrol sample for lipase production.

The phylogenetic tree shows that the bacterial isolate obtained from this study, *Bacillus megaterium* PFE2 showed a 58% percentage similarity with *Bacillus megaterium* strain JBS-19, *Bacillus aryabhattai* strain CCNWQLS38 and *Bacillus megaterium* strain KGSB16. This shows that the relationship among these four organisms is not too similar, because of the relatively low similarity percentage. There was a considerable low similarity between the bacterial isolates at the topmost part of the tree, the only exception being the similarity between *Bacillus megaterium* strain SBANST11 and the other five *Bacillus* strains at the top of the three with a similarity of 99% (accession number KR349331) MEGA5.

GENETIC SEQUENCE OF ISOLATE (PFE2) USING 16S rRNA MOLECULAR SYSTEM

AGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGAATGGTTTT
ATGGGATTGGCTTGACCTCGCGGTCTTGCAGCCCTTTGTACCATCCATTG
TAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATC
CCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTA
AATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCA
ACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTGT
CCCCCGAAGGGGAACGCTCTATCTCTAGAGTTGTCAGAGGATGTCAAGAC
CTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCT
TGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTC
CCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGCACTAAAGGGCGGAAACC
CTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTA
ATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAA
AAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCG
CTACACGTGGAATTCCGCTTTTCTCTTCTGCACTCAAGTTCCCCAGTTTC
CAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAA
CCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCT
ACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAG
GTACCGTCAAGGTACAAGCAGTTACTCTTGTACTTGTTCTTCCCTAACAA
CAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGT
CAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAG
TCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGC
TATGCATCGTTGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCAC
CGCGGGCCCATCTGTAAGTGATAGCCGAAACCATCTTTCAATCATCTCCC
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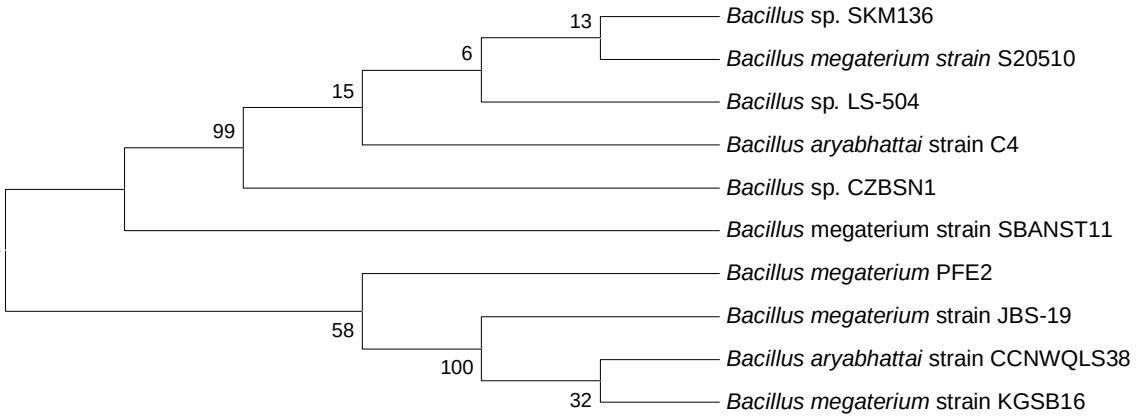

Fig. 1. Phylogenetic tree showing the evolutionary relationship of *Bacillus megaterium* PFE2 to closely related sequences obtained from the Gen Bank using Mega 5- software

3.2 Determination of Optimum pH for Lipase Production

In an attempt to determine the optimum pH for lipase production, the synthetic medium was adjusted to different pH values and incubated for 72 h. This result can be seen in Table 2. Optimum production of lipase was recorded at pH 7.0 with an activity of 2.1 U/ml at 48h. Similar observations were earlier reported by [28,29] on a *B. stearo thermophillus* that produced lipase optimally at pH 7.0 while Anurag et al. [30], reported that the optimal production of lipase by *B. megaterium* AKG-1 was at pH 7.5. These occurrences might be due to the neutral to slightly alkaline, (pH7.0-8.5) nature of culture medium for *Bacillus spp.* growth, above or below this pH the enzyme can be inactivated [2]. Sahu et al. [31] reported that changes in pH might alter the ionization of nutrient molecules and reduce

their availability to the organisms and equally, drastic variations in pH can also harm microbial cells by disrupting the plasma membrane and altering their metabolism. In addition, Kanimozhi et al. [32], observed that pH of medium influences the physiological performance of bacterial cell by facilitating the movement of growth substrate across the cell membrane which stimulates optimal production of enzyme.

Table 1. Screening of bacterial isolates from oil Press Fibre for lipolytic activities

Isolate code	Lipid zone of hydrolysis (mm)		
	24 hours	48 hours	72 hours
PFC1	2	4	4
PFD4	-	-	-
PFB3	-	-	-
PFC5	2	4	6
PFB4	4	6	6
PFB1	-	-	-
PFE2	14	18	22
PFE3	-	-	-
PFD2	6	8	8
PFA2	4	6	6
PFD1	-	-	-
PFD5	-	-	-
PFC2	-	-	-
PFC7	-	-	-
PFD3	-	-	-
PFE1	-	-	-
PFE5	6	6	8
PFB2	-	-	-
PFB5	-	-	-
PFD4	-	-	-
PFA1	2	4	6
PFA3	-	-	-
PFA5	-	-	-
PFE6	2	4	6
PFC4	-	-	-
PFC6	-	-	-
PFA3	-	-	-

Result of 16S rRNA gene sequencing

Table 2. Determination of optimum pH for lipase production

pH	24hrs	48hrs	72hrs
5	0.70±0.00^{d}	0.83±0.05^{d}	0.90±0.10^{d}
6	0.86±0.05^{c}	1.06±0.12^{c}	1.13±0.05^{c}
7	1.33±0.06^{a}	2.13±0.15^{a}	1.87±0.05^{a}
8	1.16±0.05^{b}	1.60±0.00^{b}	1.50±0.17^{b}
9	0.73±0.05^{d}	0.76±0.06^{d}	1.13±0.05^{c}

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

3.3 Determination of Optimum Temperature for Lipase Production

The optimum temperature for lipase production was determined by incubating the synthetic medium at varying temperatures for 72 h. In this study the best temperature that supported the maximum production of lipase was 35℃ with an activity of 3.3 U/ml at 72 hr while the least lipase production was observed at 25℃ with an activity of 0.87 U/ml at 24h of incubation. This result agrees with the report of Mohan et al. [33] that reported an optimum temperature for lipase production by *Bacillus* sp at 35℃ and further revealed that lipases show maximum activity at temperature range of 35 to 40℃ however, below and above this range of temperature enzymes can become inactive, denatured or destroyed due to protein denaturation beyond the optimum temperature. According to Hassan et al. [34], the difference in enzyme production at varying temperatures could be attributed to strain specificity while Ohnishi et al. [35], suggested that physical parameters such pH, temperature and location most probably exert a profound effect on lipase production by modulating growth of microorganisms. The report obtained from this study is at variance with the submissions of Sefour et al. [36] that reported a *B. stearo thermophilus* strain that produced lipase maximumly between 55 and 60℃. According Gupta et al. [2], *Bacillus* sp has the ability to grow over a wide range of temperatures between 28-60℃ and that the optimum temperature for lipase production is not the same as the growth temperature. However, it was already reported by Noman et al. [37] that low temperatures lead to gelling of plasma membrane which delays transport processes in microbial cells and results in scanty production of enzyme.

Table 3. Determination of optimum temperature for lipase production

Temperature (^{0}C)	24hrs	48hrs	72hrs
25	0.87±0.05^{c}	0.90±0.00^{c}	1.00±0.00^{c}
30	1.50±0.00^{a}	1.87±0.12^{b}	1.50±0.10^{b}
35	1.60±0.10^{a}	2.90±0.10^{a}	3.30±0.10^{a}
40	1.00±0.33^{b}	1.03±0.05^{c}	1.13±0.06^{c}

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

3.4 Effect of Different Carbon Sources on Lipase Production

In order to investigate the effect of different carbon sources on lipase production, different carbon sources were supplemented separately into the synthetic medium and incubated for 72 h. Maximum lipase production with an activity of 1.83 U/ml was recorded with the

supplementation of glucose in the medium at 72 h incubation period while the least activity of 0.76 U/ml was observed when the medium was supplemented with starch. The maximum lipase activity observed with glucose is in consonance with the earlier finding of Sharma et al. [18] who reported that some *Bacillus sp* showed higher activities when cultivated in medium containing glucose while the least activity observed with the addition of starch could be due to preferential utilization of glucose to starch which consists of complex molecules. Carbon compounds are important in the cells for provision of raw material for energy production. Each microorganism requires different utilizable carbon sources to produce lipase at its maximum level [13]

Table 4. Effect of different carbon sources on lipase production

Carbon source	24hrs	48hrs	72hrs
Starch	0.76±0.06^{d}	1.20±0.00^{d}	0.93±0.93^{c}
Sucrose	0.90±0.10^{cd}	1.00±0.10^{e}	1.33±0.12^{b}
Lactose	1.10±0.10^{bc}	1.53±0.12^{b}	1.50±0.17^{b}
Maltose	1.03±0.06^{bc}	1.36±0.06^{c}	1.50±0.10^{b}
Fructose	1.43±0.06^{a}	1.56±0.06^{b}	1.70±0.11^{a}
Glucose	1.50±0.10^{a}	1.73±0.06^{a}	1.83±0.05^{a}

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

3.5 Effect of Different Nitrogen Sources on Lipase Production

To study the effect of different nitrogen sources on lipase production in submerged fermentation, different nitrogen sources were supplemented separately into the synthetic medium and incubated for 72 h. Peptone was observed to support the maximum lipase production when compared to other nitrogen sources tested. This reported observation is in conformity with the submission of [38,39] and contrary to the earlier studies conducted by [39-42] that the addition of nitrogen sources into the production medium showed insignificant effect on the lipase production. However, the findings of [34,43] revealed that the best nitrogen source that stimulated lipase production in *B. subtilis* and *B. thermoleovorans* (HI-9) was yeast extract, while Pimentel et al. [44] reported that the replacement of yeast extract with ammonium sulphate inhibited lipase activity. According to Ghosh et al. [45], organic nitrogen sources were better than inorganic nitrogen sources such as ammonium salts, because the degradation of this salt causes acidic condition by the liberation of free fatty acid in the medium resulting in high acidic condition which may inhibit the growth of microorganism and synthesis of lipase. Nitrogen sources at high concentration had been reported to improve lipase production by microorganisms [35,46,47]. The effect of different nitrogen sources on the maximization of lipase production by microorganisms is substrate dependent which differs from one microorganism to the other.

Table 5. Effect of different nitrogen sources on lipase production

Nitrogen source	24hrs	48hrs	72hrs
Tryptone	1.16±0.05^{b}	1.53±0.05^{b}	2.06±0.05^{b}
Yeast extract	1.00±0.00^{c}	1.10±0.10^{c}	1.93±0.12^{b}
Peptone	1.67±0.05^{a}	2.33±0.05^{a}	2.60±0.10^{a}
KNO_3	0.80±0.00^{d}	0.76±0.05^{d}	1.03±0.05^{d}
$(NH_4)_2SO_4$	1.03±0.05^{c}	1.16±0.05^{c}	1.70±0.10^{c}

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

3.6 Determination of Optimum Incubation Period for Lipase Production

The optimum incubation period for lipase production was monitored by incubating the synthetic medium for different periods. Maximum lipase production was observed at 72 h (Fig. 2). However, Sekhon et al. [27] reported that maximum production of lipase by *Bacillus stearo thermophillus* occurred at 24 hr of incubation which is inconsistent with the findings of [29] that discovered that *B. stearo thermophillus* produced optimum lipase at 30h of incubation, while [48] reported maximum production of extra celluar lipase by *B. megaterium* AKC-1 at 34 h of investigation. Rehman et al. [38] reported that maximum production of extracellular lipase by *Penicillum notatum* occurred *at* 96 h of incubation after which it decreased probably due to depletion of nutrients and denaturation of the enzyme resulting from the interaction with other components in the production medium or alteration in the pH of the medium [49]. Summarily, the results stated above showed that incubation periods for maximum production of lipase vary from one microorganism to the other.

3.7 Effect of Different Ions on Lipase Production

To verify the effect of different anions on lipase production, various anions were supplemented differently at 1% concentration into the synthetic medium and incubated for 72 h. The result

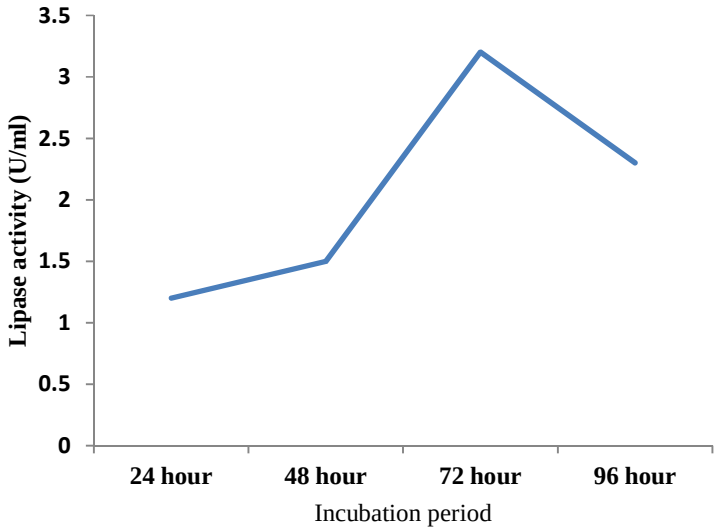

Fig. 2. Determination of optimum incubation Period for lipase production

(Table 7) showed that the highest lipase production with an activity of 1.86±0.05 was obtained with the addition of 0.3M chloride ion to the growth medium while the lowest production of lipase with an activity of 1.03±0.05 was recorded when 0.3M OH^- was substituted. The effect of different concentrations of cations (Na^+, K^+, Mn^{2+},Ca^{2+} and Mg^{2+}) was investigated on lipase production, Ca^{2+} at 0.3M concentration stimulated the highest lipase production with an activity of 2.40±0.10u/ml, while the lowest activity of 0.96±0.05 u/ml was recorded when 0.2 M of K^+ was introduced into the growth medium (Table 8). Mahanta et al. [49] reported a lipase from *Bacillus subtilis* with improved activity when incubated in 10mM Ca^{2+}.while Kambourova et al.[50] reported that extracellular lipase produced by *Bacillus sp* showed complete activity in the presence Ca^{2+} and Ca^{2+} has been found to stimulate enzyme activity due to the formation of calcium salts of long chain fatty acids [51]. In addition, divalent cation influences the enzyme activity depending on the temperature at which protein-ion metal interaction occurred.

Table 7. Effect of different concentrations of anions on lipase production

Anions	0.1M	0.2M	0.3M
SO_4-	1.36±0.05^c	1.76±0.05^a	1.43±0.05^b
OH^-	1.73±0.05^a	1.33±0.12^b	1.03±0.05^c
NO_3-	1.40±0.00^c	1.06±0.05^c	1.06±0.05^c
Cl^-	1.60±0.00^b	1.80±0.00^a	1.86±0.05^a

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

Table 8. Effect different concentrations of cations on lipase production

Cations	0.1M	0.2M	0.3M
Na^+	1.80±0.05^a	1.70±0.05^b	1.10±0.05^d
K^+	1.23±0.05^c	0.96±0.05^d	1.23±0.05^{cd}
$Ca2^+$	1.43±0.05^b	1.73±0.50^a	2.40±0.10^a
Mn^{2+}	1.43±0.12^b	1.33±0.05^c	1.33±0.05^{bc}
Mg^{2+}	1.26±0.05^c	1.60±0.00^b	1.36±0.05^b

Values in the same column followed by the same letter are not significantly different according to Duncan's multiple range test (p<0.05)

4. CONCLUSIONS

This study showed that lipase production by *B. megaterium* can be optimized and the best conditions for optimization included pH 7.0, temperature of 35℃, 72 hours incubation period in the presence of 2% glucose, 2.5% peptone concentrations and 0.3M Ca^{2+} and 0.3M Cl^-.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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